ORIGINAL PAPERS

Hypoproteinemia and alpha globulin deficiency in round heart disease of turkeys

RUTH MEIROM, MSc, Z. TRAININ, Dr med vet, ADA BARNEA, BSc, F. NEUMANN, Dr med vet, PhD, U. KLOPFER, Dr med vet, T. A. NOBEL, Dr med vet, DTVM, Kimron Veterinary Institute, Bet Degan, Israel
M. S. DIXON, BA, BVSc, District Poultry Laboratory, Hadera. O. PLESSER, DVM, District Poultry Laboratory, Jerusalem

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SUMMARY.—Turkeys affected with round heart disease had a marked reduction in total serum protein. While the normal mean value was 3·8 ± 0·4g per 100 ml, the total serum protein value of the turkeys with round heart disease was 2·1 ± 0·7 g per 100 ml. The immunoelectrophoretic analyses in 87 per cent of the affected sera revealed a characteristic alpha globulin deficiency. The comparison between this phenomenon and alpha-1 antitrypsin deficiency in man is discussed.

Introduction

THE clinical and pathological aspects of round heart disease (RHD) in turkeys were reviewed by Neumann, *et al* (1973). The same authors described specific intracytoplasmic liver cell globules in the above condition, and emphasised the similarity between these globules and those found in alpha-1 antitrypsin deficiency in man, as described by: DeLellis, *et al* (1972); Gordon, *et al* (1972); Lieberman, *et al* (1972); Campra, *et al* (1973); Glasgow, *et al* (1973). This condition in man is considered to be hereditary. Because of similarity of hepatic lesions it was hypothesized that RHD of turkeys may be related etiologically to the above condition in man. Therefore, a study of the serum proteins of turkeys was undertaken to denote if changes similar to those found in man affected with alpha-1 antitrypsin deficiency, occur in turkeys with RHD.

Materials and methods

Complete necropsies were performed on 62 Nicholas turkeys with RHD, aged eight to 12 weeks, mostly males and on 123 healthy Nicholas turkeys aged 10 weeks. Forty-two of the RHD affected turkeys were dead when tested, all the other birds were slaughtered. For histological purposes representative pieces of liver were removed and fixed in 10 per cent neutral formalin. Periodic acid Schiff (PAS) reactions were completed on 7μ paraffin sections, to detect the presence of PAS positive intracytoplasmic liver cell globules as described by Neumann, *et al* (1973) in RHD of turkeys.

An enlarged and dilated heart, as well as PAS positive globules in the liver cells were considered the criteria for a diagnosis of RHD and were used as a preliminary screening prior to the blood analyse.

Blood examinations

All blood samples were taken from the heart. The blood clots were removed on the same day as procured and the sera were stored at −20° C until tested.

Total protein estimation of serum was carried out by Biuret's method as described by Fey, *et al* (1964).

Antisera Anti-fowl and anti-turkey serum were produced by injecting rabbits with 0·1 ml of fowl serum and turkey serum respectively, with complete Freund adjuvant in the footpad. Boosters of 0·1 ml of serum were given intramuscularly three times at weekly intervals. The animals were bled a week after the last booster and the anti-fowl rabbit sera and anti-turkey rabbit sera were stored at −20° C until used.

Immunoelectrophoresis was carried out for all the tested sera as described by Trainin (1969). The antisera used were anti-fowl and anti-turkey rabbit sera.

Negative immunoelectrophoresis To determine proteins which might be lacking in the serum of affected turkeys, the following procedure was used. The electrophoresis was carried out with normal control serum, but before adding antiserum, 0·1 ml of the tested serum was put in the trough for five minutes and the antiserum was added as in regular immunoelectrophoresis. The antibodies which were not absorbed by the tested serum could react with the normal serum. Thus the precipitating lines represent only the proteins which are absent in the tested serum.

Results

Total serum protein in normal turkeys and with RHD

The sera of 47 healthy turkeys aged 10 weeks and the sera of 25 turkeys affected with RHD of the same age were tested in order to determine the total serum protein level. The mean value of the normal turkeys was 3·8 ± 0·4 (s.d.) g per 100 ml, while that of the affected ones was 2·1 ± 0·7 (s.d.) g per 100 ml. The variance of the affected birds was higher significantly ($p < 0.01$) than that of the healthy ones, as some of the affected turkeys had serum protein levels approximating that of the healthy ones. The means were compared accordingly, by the method suggested by Cochran and Cox (1957) and were found to differ significantly ($p < 0.01$). Hypoproteinemia in the

serum of most of the affected turkeys could be seen clearly in the immunoelectrophoresis (Fig. 1).

Immunoelectrophoretic analyses

Immunoelectrophoretic tests of the sera of the affected turkeys (RHD) revealed a partial reduction of almost all serum proteins and a complete lack of one to two precipitating lines in the alpha globulin line (Figs 1 and 2), sometimes associated with the lack of the beta globulin line (Fig 2). One more precipitating line in the alpha region could be seen by the negative immunoelectrophoresis of the same sera. This line normally occluded by the albumin.

All 20 (100 per cent) slaughtered birds which were affected lacked alpha globulins in their sera. From 42 affected turkeys which were dead, 34 (81 per cent) also lacked the above mentioned globulin. In eight turkeys of the last group alpha globulins were present in their sera. Alpha globulin deficiency could be observed also in those affected birds which had normal total serum protein values.

In all, 54 affected turkeys (RHD) out of 62 (87 per cent) had an alpha globulin deficiency, as determined in the direct or negative immunoelectrophoretic tests. This deficiency was not observed in 123 healthy turkeys.

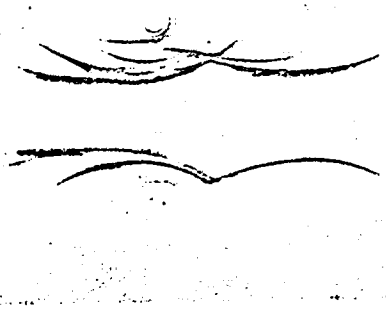

FIG 1: Immunoelectrophoretic analysis of turkey's sera. *Above*: Serum of 10 weeks old healthy turkey. *Below*: Serum of 10 weeks old turkey with Round Heart Disease. *Trough*: Rabbit anti turkey serum.

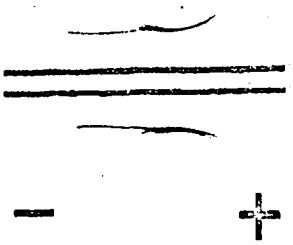

FIG 2: Negative immunoelectrophoretic analysis of turkey's sera. *Above and below*: Serum of 10 weeks old healthy turkey. *Trough*: Rabbit anti turkey serum absorbed with serum of 10 weeks old turkey affected with Round Heart Disease.

Comparative studies between serum and liver changes

To assess the relationship between a deficiency of alpha globulins and the concomitant present of cytoplasmic liver cell globules, a comparative study was completed. Forty-five RHD positive birds out of 62 revealed typical liver changes and an alpha globulin deficiency. In the remaining 17 cases either serum or liver changes occurred alone. Eight of nine birds with alpha globulin deficiency and without the intracytoplasmic liver cell globules were slaughtered.

Discussion

The total protein levels in the sera of the 10-week-old healthy turkeys are similar to those found by Bierer (1969). According to Clarkson (1966) and Bierer (1969) there are no marked differences among the protein levels of six to 12-week-old turkeys. The mean level of the serum protein of the RHD affected turkeys is significantly reduced, approximately 50 per cent. However, the variation in the levels among the affected turkeys is wide and some of the birds have protein values in the normal range. The possibility exists that the general reduction of the serum protein results from hepatic damage.

Round heart disease appears to be characterised by an alpha globulin deficiency. Such an alteration was not observed among the 123 healthy turkeys, but was seen in 87 per cent of the RHD affected turkeys. The fact that this alteration occurred also in turkeys with total protein levels in the normal range, suggests that an alpha globulin deficiency may be independent of the total protein reduction.

The comparative studies between typical serum and liver changes suggested that alpha globulin deficiency might occur before the intracytoplasmic liver cell globules are visible. The fact that these globules were not found in eight of 20 slaughtered turkeys with RHD, but were present in all dead birds, is similar to the observations of Gordon, *et al* (1972) in man. More intracytoplasmic liver cell globules were found in material obtained at autopsy than from hepatic biopsies obtained from individuals acutely ill.

Although, the difference between the percentage of slaughtered and dead affected birds having alpha globulin deficiency is not significant, it appears that serum values of liver of slaughtered birds are more representative than those of dead birds. The hypothesis that the proteins which are absent from the serum are produced, but blocked in the liver, as in alpha-1 antitrypsin deficiency in man (Gordon, *et al*, 1972) needs further investigation. In the event that the hypothesis will be proven, the possibility exists that the autolysis of liver cells after death may release these proteins to the serum and thus cause false "negative" reactions. This study has shown that immunoelectrophoretic analyses and especially the negative immunoelectrophoretic method can be used diagnostically in the detection of RHD affected turkeys. The alpha globulin which is normally invisible because it is masked by the albumin fraction resembles that of alpha-1 antitrypsin in man, in the immunoelectrophoretic picture (Erikson, 1965).

The specific identification of the deficient alpha

globulin in the sera of RHD affected turkeys and the relationship, if any, to antitrypsin, needs further investigations.

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RÉSUMÉ. Les dindons affectés avec la Maladie du Coeur Rond, demontrent une réduction importante de la proteine totale du serum. Tandis que, les valeurs moyens normaux sont de 3·8 ± 0·4 g pour 100 ml la proteine totale du serum chez les dindons affectee de la Maladie du Coeur Rond est de 2·1 ± 0·7 g pour 100 ml. Les analyses immunoelectrophoretiques montrent dans 87 pour cent des sera provenant des dindons atteints, une déficience characteristique des alpha globulins. La comparaison entre ce phénoméne et la alpha-1 antitrypsine déficience de l'homme est discutée.

ZUSAMMENFASSUNG. Die von Rundherzkrankheit befallenen Puten zeigen eine bedeutende Verringerung des totalen Proteins im Blutserum auf. Während die normalen Mittelwerte 3·8 ± 0·4 g pro 100 ml betragen, ist der totale Proteinwert des Blutserums bei mit Rundherzkrankheit befallenen Puten 2·1 ± 0·7 g pro 100 ml. Die immunoelektrophoretischen Analysen zeigen in 87 prozent der von kranken Puten untersuchten Sera einen charakteristischen Ausfall der Alpha Globuline. Der Vergleich zwischen diesem Phänomen und dem Alpha-1 Antitrypsinausfallides Menschen wird besprochen.

Ovine paralysis associated with spinal lesions of toxoplasmosis

B. A. McERLEAN, MVM, MRCVS, Department of Veterinary Medicine, Pharmacology and Food Hygiene, University College Dublin, Ireland

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SUMMARY.—Two Cheviot sheep, six months of age, developed a progressive paralysis of the limbs shortly after purchase. They had a persistent fever and elevated leucocyte counts. Extensive perivascular cuffing was found in the histological sections of the spinal cord and several *Toxoplasma* pseudocysts were seen.

Introduction

WHILE toxoplasmosis has been detected in many species of animals often in a latent form, it is of particular significance in sheep as in this species it is responsible for outbreaks of abortion and perinatal mortality. Crowley (1964) in Ireland described one outbreak where elevated serum titres were obtained following abortions. Apart from abortions and perinatal mortality there are very few reported cases of other clinical forms of toxoplasmosis in the adult sheep.

Olafson and Monlux (1942) in the United States, described a case in a ewe in which there were nervous signs, difficulty in moving, paresis and dyspnoea. Histological examination revealed an acute diffuse non-suppurative encephalomyelitis and *Toxoplasma* cysts were found in the brain. Wickham and Carne (1950) reported a similar case in Australia. Cole, *et al* (1954) in the U.S.A., recorded the deaths of five ewes and three lambs with signs of disease in the respiratory and central nervous systems. The only case recorded in the United Kingdom involved a two-year-old ewe in which encephalitic symptoms were seen (Zlotnik, 1959).

The purpose of this report is to record the first outbreak of clinical toxoplasmosis in adult sheep in Ireland.

Clinical report

A farmer living in the Dublin Mountains bought 40 six-month-old Cheviot sheep from a neighbour early in October, 1971. Two days later one sheep was unable to rise but got up when helped. A few days later it was unable to stand. Ten days later a second sheep began to sway on the hindquarters. On October 27, 1971 (Day 0) these two affected sheep were brought to the University College Dublin veterinary hospital. The first affected sheep (A) was paralysed in all four limbs. The second sheep (B) was able to stand but swayed unsteadily on the hind legs and resembled a case of sway-back. Both sheep were bright and showed no encephalitic symptoms. They had good appetites and ruminated frequently. Both animals had temperatures between 104·5 to 105·0 F until they were killed. Several blood samples were taken and the findings are indicated in Table I. Radiographs were taken of the dorsal and lumbar spinal regions of each sheep. These failed to reveal any lesions. Each sheep received 2·5 ml of tetracycline* daily, commencing on Day 2. Sheep A received [illegible] injections and was killed on Day 6. Sheep B received several injections; however, it gradually

* Rexocin—Hoechst Pharmaceuticals